Benchmarks

Direct PCR amplification and sequencing of specimens' DNA from preservative ethanol

Shadi Shokralla, Gregory A. C. Singer, and Mehrdad Hajibabaei
Biodiversity Institute of Ontario, Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada

BioTechniques 48:XXX-XXX (March 2010) doi 10.2144/000113362
Keywords: DNA extraction, Sample preparation, Biodiversity, DNA barcoding, Museum samples

Supplementary material for this article is available at www.BioTechniques.com/article/113362.

DNA extraction is the first step in many molecular biology protocols. However, we hypothesized that DNA from a preserved specimen can leak into its preservative medium, allowing the medium itself to be directly PCR amplified. We successfully tested this idea on mescal—the alcoholic beverage famous for the "worm" (a caterpillar) that is placed in the bottle of many brands—and indeed obtained amplifiable quantities of caterpillar DNA. We then successfully amplified and sequenced DNA from the 95% ethanol preservative of 70 freshly collected specimens and 7 archival specimens 7–10 years old. These results suggest that DNA extraction is a superfluous step in many protocols and that preservative ethanol can be used as a source of genetic material for non-invasive sampling or when no tissue specimen is left for further DNA analyses.

DNA extraction is the first step in many molecular biology experiments, but is a process that has not been significantly altered for over twenty years (1). It is considered necessary in order to release sufficient amounts of DNA for downstream applications such as PCR, cloning, and DNA sequencing. Therefore, a number of commercial kits have been developed to extract DNA from different tissue types and specimens. These procedures force cells to release their DNA via physical perturbation and/or chemical treatment, which is then followed by a clean-up procedure in which unwanted cellular components are separated from the DNA (2). Some simplified protocols use capture resins such as Chelex or alkaline lysis for rapid DNA isolation (2). However, modern PCR enzymes are so robust that we wondered whether the DNA extraction step was necessary for biological specimens preserved in solution. We hypothesized that a small amount of DNA leaks from the tissue into the preservation solution (usually ethanol), and that this DNA was amplifiable using a standard PCR protocol. Thus, we reasoned, the preservative ethanol could be used as a source of genetic material for non-invasive analyses (e.g., for type specimens) or when DNA analyses are required for specimens that have been consumed in prior experiments. A recent study showed that an organism's DNA can be detected from the water in which it lives (3); however, this study still employed DNA extraction using a commercially-available kit.

In order to test our idea, we initially used a popular example: mescal, the tequila-like liquor containing a "worm" (the larvae of the agave butterfly, *Hypopta agavis*). Fifty milliliters of Monte Alban mescal was evaporated in an incubator at 56°C and the residue was dissolved in 50 µL molecular biology grade water. This solution was passed through a Qiagen MinElute spin column to remove PCR inhibitors and the DNA was then re-dissolved in 50 µL water. In parallel, we applied a similar approach to freshly collected and archival specimens stored in 95% ethanol. The fresh specimens were placed in 2 mL 95% ethanol (whole insects and 0.5-mm tissue samples of plant leaf). After 24 h, we transferred 1mL ethanol from the tubes containing specimens to a new tube and let the ethanol evaporate at 56°C (~30 min). For the older specimens, we used 1 mL of the preservative ethanol as starting material to evaporate at 56°C. We dissolved the residual DNA in 30 µL of molecular biology grade water. In all samples, we used a 2-µL aliquot of DNA solution for subsequent PCR amplification.

We amplified 130-base and 658-base fragments of cytochrome C oxidase 1 (*CO1*) for the mescal sample and other animals using standard DNA barcoding protocols and primers (4–7). We also amplified nuclear ribosomal 28S rDNA (8) for freshly collected insect specimens and a partial fragment of *rbcL* (9) gene for all plant specimens (Supplementary Table S1). We performed PCR using premixed PCR plates employing Platinum Taq polymerase (Invitrogen, Burlington, ON, Canada). Sequences were obtained using an ABI 3730xl sequencer (Applied Biosystems, Foster City, CA, USA) and edited by the CodonCode aligner software, version 3.0.3 (Dedham, MA, USA). Detailed PCR and sequencing conditions are available in the Supplementary Material.

DNA from mescal was successfully PCR-amplified and sequenced (Figure 1). For comparison purposes, genomic DNA was extracted from the caterpillar tissue using the Nucleospin Tissue Kit (Macherey-Nagel, Düren, Germany) and the 130-base *CO1* mini-barcode was then amplified and sequenced (Figure 1). Comparison to an existing library of Lepidoptera DNA barcodes at BOLD (10) confirmed that we had sequences related to the family Cossidae, to which the agave butterfly belongs. This is a surprising result since mescal is an imperfect preservation medium: it is only 40% ethanol and contains many impurities that can degrade DNA. Additionally, the worm's body was completely intact with no obvious external damage that could have released DNA in large quantities. Although we were not able to amplify a full-length DNA barcode (i.e., 650 bases) from the mescal, we did amplify this fragment from a tissue sample obtained from the worm, indicating that the DNA obtained from the mescal had been released from the worm body and had degraded over time (11).

We repeated this approach on a variety of freshly collected specimens including whole insects (caddisflies and mayflies) and plant leaves. Using DNA directly obtained

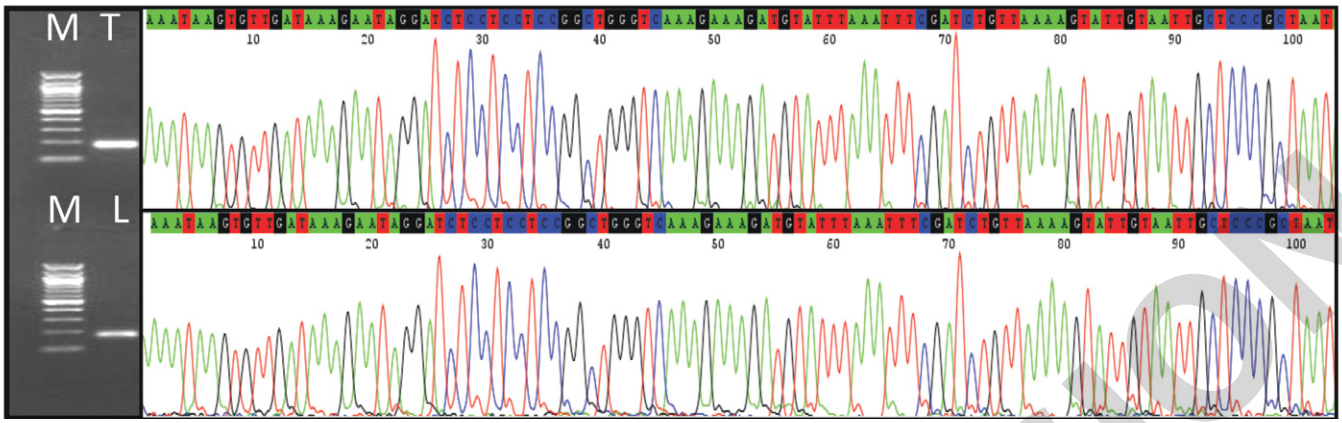

Figure 1. DNA leaking from specimens in preservative liquids can be directly used for PCR and sequencing. (Left) Gels showing the 130 base–long amplicon from the cytochrome c oxidase 1 (*CO1*) gene from DNA extracted from caterpillar tissue specimen (T), compared with the same amplicon obtained directly from mescal liquid (L). M, molecular size marker. (Right) Corresponding sequencing electropherogram of the PCR amplicon shown at left.

from the 95% ethanol preservative resulted in 25/25 and 24/25 sequencing success for animal *CO1* and 28S rDNA, respectively, as well as 45/45 sequencing success for plant *rbcL* (Supplementary Table S1). We also successfully applied this approach to seven specimens from a variety of different phyla that were collected 7–10 years ago and have been preserved at room temperature in 95% ethanol since the time of collection (Supplementary Table S1). These results suggest a wide applicability of the approach: preservative-derived DNA from tissue specimens (such as pieces of plant leaf), intact organisms (such as the caterpillar in mescal or whole mayflies and caddisflies), and older archival specimens from a wide variety of taxa worked equally well. In addition, the DNA from 95% ethanol preservative universally produced large amplicons (i.e., 650 bases of the *CO1* gene), derived from all three genomic compartments (nuclear, mitochondrial, and plastid).

Other studies—especially non-invasive extraction protocols or those for use with ancient DNA—have shown that DNA is released from tissue samples and can be captured or purified for downstream applications (12,13). Our results reinforce these observations but also provide ample evidence that DNA capture is not needed since residual DNA in the medium is robustly amplifiable by a simple PCR reaction, even in the case of specimens stored under less-than-ideal conditions. This result is especially important for developing inexpensive and high-throughput non-invasive genetic analyses where preservation of the original tissue is required or when there is simply no sample left for further analysis—a situation frequently encountered by researchers. Coupled with whole-genome amplification protocols, our approach can potentially provide larger quantities of DNA. Our results also suggest that field sampling procedures that include placing mixtures of specimens in an ethanol jar should be avoided, since residual DNA from all specimens is present in the ethanol preservative and may increase the chance of cross-contamination.

Acknowledgements

We thank Donal Hickey for providing the mescal and for helpful discussions, Maria Kuzmina for helping to collect and identify plant samples, Xin Zhou for assistance in sampling and identifying insects, Alexey Borisenko and Jamye Sones for assistance in locating archival specimens, and Barton Slatko for reviewing an earlier version of the manuscript. This study was supported by funds from Genome Canada through the Ontario Genomics Institute.

Competing interests

The authors declare no competing interests.

References

1. **Sambrook, J. and D. Russell.** 2001. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
2. **Hajibabaei, M., J.R. deWaard, N.V. Ivanova, S. Ratnasingham, R.T. Dooh, S.L. Kirk, P.M. Mackie, and P.D.N. Hebert.** 2005. Critical factors for assembling a high volume of DNA barcodes. Philos. Trans. R. Soc. Lond. B Biol. Sci. *360*:1959-1967.
3. **Ficetola, G.F., C. Miaud, F. Pompanon, and P. Taberlet.** 2008. Species detection using environmental DNA from water samples. Biol. Lett. *4*:423-425.
4. **Hajibabaei, M., D.H. Janzen, J.M. Burns, W. Hallwachs, and P.D.N. Hebert.** 2006. DNA barcodes distinguish species of tropical Lepidoptera. Proc. Natl. Acad. Sci. USA *103*:968-971.
5. **Meusnier, I., G.A. Singer, J.F. Landry, D.A. Hickey, P.D.N. Hebert, and M. Hajibabaei.** 2008. A universal DNA mini-barcode for biodiversity analysis. BMC Genomics *9*:214.
6. **Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek.** 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. *3*:294-299.
7. **Hebert, P.D.N., A. Cywinska, S.L. Ball, and J.R. deWaard.** 2003. Biological identifications through DNA barcodes. Proc. Biol. Sci. *270*:313-321.
8. **Saux, C., B.L. Fisher, and G.S. Spicer.** 2004. Dracula ant phylogeny as inferred by nuclear 28S rDNA sequences and implications for ant systematics (Hymenoptera: Formicidae: Amblyoponinae). Mol. Phylogenet. Evol. *33*:457-468.
9. **Levin, R.A., W.L. Wagner, P.C. Hoch, M. Nepokroeff, J.C. Pires, E.A. Zimmer, and K.J. Sytsma.** 2003. Family-level relationships of Onagraceae based on chloroplast rbcl and ndhf data. Am. J. Bot. *90*:107-115.
10. **Ratnasingham, S. and P.D.N. Hebert.** 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Mol. Ecol. Notes *7*:355-364.
11. **Zimmermann, J., M. Hajibabaei, D.C. Blackburn, J. Hanken, E. Cantin, J. Posfai, and T.C. Evans, Jr.** 2008. DNA damage in preserved specimens and tissue samples: a molecular assessment. Front. Zool. *5*:18.
12. **Asher, R.J. and M. Hofreiter.** 2006. Tenrec phylogeny and noninvasive extraction of nuclear DNA from museum specimens. Syst. Biol. *55*:181-194.
13. **Phillips, A.J. and C. Simon.** 1995. Simple, efficient, and nondestructive DNA extraction protocol for Arthropods. Ann. Entomol. Soc. Am. *88*:281-283.

Received 20 August 2009; accepted 11 January 2010.

Address correspondence to Mehrdad Hajibabaei, Biodiversity Institute of Ontario, Department of Integrative Biology, University of Guelph, Guelph, Ontario, N1G 2W1 Canada. e-mail: mhajibab@uoguelph.ca